

Isolation of Bacterial Plasmid-Related Replication-Associated Circular DNA from a Serum Sample of a Multiple Sclerosis Patient

Karin Gunst, Harald zur Hausen, Ethel-Michele de Villiers

Division for the Characterization of Tumorviruses, Deutsches Krebsforschungszentrum, Heidelberg, Germany

***Psychrobacter* species are considered to be opportunistic human pathogens. We report here the isolation of a circular DNA molecule, MSS11.162, from a serum sample taken from a multiple sclerosis patient during relapse. This isolate is distantly related to known *Psychrobacter* species and their plasmids.**

Received 28 July 2014 Accepted 4 August 2014 Published 28 August 2014

Citation Gunst K, zur Hausen H, de Villiers E-M. 2014. Isolation of bacterial plasmid-related replication-associated circular DNA from a serum sample of a multiple sclerosis patient. *Genome Announc*. 2(4):e00847-14. doi:10.1128/genomeA.00847-14.

Copyright © 2014 Gunst et al. This is an open-access article distributed under the terms of the [Creative Commons Attribution 3.0 Unported license](https://creativecommons.org/licenses/by/3.0/).

Address correspondence to Ethel-Michele de Villiers, e.devilliers@dkfz.de.

Psychrobacter species are frequently present as food contaminants and have been isolated from human tissues, including the brain, cerebrospinal fluid, and blood. They are considered to be opportunistic human pathogens (1, 2). We report here the isolation of a circular DNA molecule, MSS11.162, from a serum sample taken from a multiple sclerosis patient during relapse.

Rolling circle amplification and restriction digestion (3) were performed on DNA extracted from serum samples from patients with multiple sclerosis. The resulting fragment was cloned into vector pUC19. The full-length genome was verified by inverted PCR amplification using primers designed on the sequence identified initially: forward primer 5'-GACTTCTGATTGATTGATGCTTG-3' and reverse primer 5'-CCTGTTGAATACCGCTTAAATACT-3'. All products were sequenced by primer walking. MSS11.162 (MSSI, multiple sclerosis serum isolate) (1,627 bp) shares 66% nucleotide similarity by BLASTn analysis with the pRWF102 plasmid of *Psychrobacter* sp. PRwf-1. The putative protein (321 amino acids) encoded by the large open reading frame (ORF) shows weak similarity to the replication protein family of *Escherichia coli*, as analyzed by ProtSweep (4), and it has 60% similarity to the Rep protein of *Psychrobacter* sp. PRwf-1 plasmid pRWF102.

This new isolate is only distantly related to known *Psychrobac-*

ter species and their plasmids. It may therefore represent a yet unknown human pathogen.

Nucleotide sequence accession number. The complete sequence of MSS11.162 is available in the EMBL Databank under the accession no. [LK931486](https://www.ebi.ac.uk/ena/record/LK931486/).

ACKNOWLEDGMENTS

This study was supported in part by Oryx, München, Germany.

We thank Imke Grewe and Sonja Stephan for their excellent technical assistance.

REFERENCES

1. Lloyd-Puryear M, Wallace D, Baldwin T, Hollis DG. 1991. Meningitis caused by *Psychrobacter immobilis* in an infant. *J. Clin. Microbiol.* 29: 2041–2042.
2. Caspar Y, Recule C, Pouzol P, Lafeuillade B, Mallaret MR, Maurin M, Croize J. 2013. *Psychrobacter arenosus* bacteremia after blood transfusion, France. *Emerg. Infect. Dis.* 19:1118–1120. <http://dx.doi.org/10.3201/eid1907.121599>.
3. Funk M, Gunst K, Lucansky V, Müller H, zur Hausen H, de Villiers E-M. 2014. Isolation of protein-associated circular DNA from healthy cattle serum. *Genome Announc* 2(4):e00846-14. <http://dx.doi.org/10.1128/genomeA.00846-14>.
4. del Val C, Ernst P, Falkenhahn M, Fladerer C, Glatting KH, Suhai S, Hotz-Wagenblatt A. 2007. ProtSweep, 2DSweep and DomainSweep: protein analysis suite at DKFZ. *Nucleic Acids Res.* 35:W444–W450. <http://dx.doi.org/10.1093/nar/gkm364>.